SUPPORTING INFORMATION

Polysaccharide succinylation enhances the intracellular survival of Mycobacterium abscessus

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<u>Table S1</u>: Monosaccharidic composition of LM and LAM from WT *Mabs* ATCC 19977, the Δ*sucT* mutant and the complemented mutant strain.

Reported values are averages \pm standard deviations of three technical repeats and represent relative distribution in %.

(A) Monosaccharidic composition of LAM

	Araf	Ino	Manp	2-O-methyl-Manp	Araf/Manp
WT	78.0 ± 1.2	0.5 ± 0.1	20.3 ± 1.4	1.2 ± 0.3	3.6 ± 0.3
$\Delta sucT$	81.3 ± 1.1	0.5 ± 0.1	17.1 ± 1.3	1.0 ± 0.2	4.5 ± 0.4
$\Delta sucT \operatorname{comp}$	78.2 ± 0.6	$0.4\ \pm 0.1$	19.9 ± 0.6	1.5 ± 0.2	3.7 ± 0.1

The LAMs produced by the three strains show no statistically significant difference in Araf/Manp ratio pursuant to the Student's t-test (P < 0.05).

(B) Monosaccharidic composition of LM

	Ino	Manp	2-O-methyl-Manp
WT	1.5 ± 0.5	95.6 ± 1.1	2.9 ± 0.7
$\Delta sucT$	1.5 ± 0.6	95.1 ± 0.5	3.4 ± 0.7
$\Delta sucT \operatorname{comp}$	2.3 ± 0.5	94.6 ± 0.6	3.1 ± 0.2

<u>Table S2</u>: Glycosyl linkage analysis of per-O-methylated LM and LAM from WT *Mabs* ATCC 19977, the $\Delta sucT$ mutant and the complemented mutant strain.

Reported values are averages \pm SD of three technical repeats and represent relative distribution in %.

(A)	Gl	ycosy	'l link	age an	alysis	of per-	O-methv	ylated L	AM^{a}

	t-Araf	2-Araf	5-Araf	3,5-Araf	t-Manp	6-Manp	3,6-Manp ^b	Araf/Manp	3,6-Manp/6-Manp	2-Araf/5-Araf
WT	9.5 ± 1.0	6.4 ± 0.3	45.8 ± 0.8	14.7 ± 0.5	10.1 ± 0.7	7.2 ± 0.3	6.3 ± 0.1	3.1 ± 0.1	0.9	0.14 ± 0.01
$\Delta sucT$	10.2 ± 1.3	8.2 ± 0.5	46.0 ± 0.8	11.7 ± 1.1	13.9 ± 0.4	5.1 ± 0.5	4.8 ± 0.3	4.2 ± 0.5	0.9	0.18 ± 0.01
$\Delta sucT$ comp	14.0 ± 1.2	8.1 ± 0.9	37.0 ± 1.1	14.6 ± 0.5	10.3 ± 0.2	7.5 ± 0.5	8.5 ± 1.2	2.7 ± 0.3	1.1	0.22 ± 0.02

^aThe LAM produced by the three strains show no statistically significant differences in terms of glycosyl linkages of their mannan and arabinan domains pursuant to the Student's *t*-test (P < 0.05). ^b2,6 linked Manp was found in small variable amounts (less than 16% of the 3,6-Manp). We attribute this to under methylation as 2,6-Manp could not be detected in the NMR analysis.

(B) <u>Glycosyl linkage analysis of per-O-methylated LM^a</u>

	t-Manp	6-Manp	3,6-Manp ^b	3,6-Manp/6-Manp
WT	52.3 ± 3.7	20.5 ± 1.7	27.2 ± 2.7	1.3
$\Delta sucT$	55.5 ± 1.0	18.0 ± 1.3	26.5 ± 0.7	1.5
$\Delta sucT \operatorname{comp}$	46.0 ± 1.0	20.4 ± 0.6	33.6 ± 0.9	1.6

^aThe LM produced by the three strains show no statistically significant differences in terms of glycosyl linkages of their mannan domain pursuant to the Student's *t*-test (P < 0.05). ^b2,6 linked Man*p* was found in small variable amounts (less than 16% of the 3,6-Man*p*). We attribute this to under methylation as 2,6-Man*p* could not be detected in the NMR analysis.

<u>Table S3</u>: Fatty acid composition of the mannosylated phosphatidyl-*myo*-inositol anchor of LM and LAM from WT *Mabs* ATCC 19977 and the $\Delta sucT$ knockout mutant.

						C19:0
	C14:0	C15:0	C16:0	C18:1	C18:0	(TBSA)
LM WT	0.9	0.7	36.20	6.40	32.9	22.9
LM $\Delta sucT$	1.2	0.4	34.6	1.6	45.8	16.4
LAM WT	14.2	1.5	66.1	1.1	9.1	8.0
LAM $\Delta sucT$	9.0	1.5	57.1	4.8	20.6	7.0

Reported values represent relative distribution in %. TBSA: Tuberculostearic acid.

	WT	$\Delta sucT$	$\Delta sucT \operatorname{comp}$
Total Ara ₄	50.2	38.6	52.4
Unmodified Ara ₄	77	95.8	80.8
Ara ₄ +succinate	14.5	0	13.4
Ara ₄ +acetate	7.6	4.2	5.2
Ara ₄ +succinate+acetate	0.9	0	0.6
Total Ara ₆	49.8	61.4	47.6
Unmodified Ara ₆	86.9	98.4	88.3
Ara ₆ +succinate	5.8	0	6.6
Ara ₆ +acetate	7.3	1.6	5.0
Ara ₆ +succinate+acetate	0	0	0.1
SUM	100	100	100

<u>Table S4</u>: Relative percentage of Ara₄ and Ara₆ oligoarabinosides released upon *Cellulomonas gelida* endoarabinanase digestion and the individual representation (%) of covalently modified oligoarabinosides within each group.

<u>Table S5</u>: Monosaccharidic composition of mAGP from WT *Mabs* ATCC 19977, the $\Delta sucT$ mutant and the complemented mutant strain. Reported values are averages \pm SD of three technical repeats and represent relative distribution in %. No statistically significant differences between strains were observed pursuant to the Student's *t*-test (P < 0.05).

	Rha <i>p</i>	Araf	Galf	GlcNAc	MurNAc	Araf/Galf	Araf/Rhap	Galf/Rhap	mycolic acids/Rhap
WT	1.1 ± 0.1	46.0 ± 2.3	22.5 ± 2.0	10.5 ± 1.2	15.2 ± 3.3	2.1 ± 0.1	43.0 ± 5.1	20.9 ± 2.8	25.4 ± 9.8
$\Delta sucT$	1.2 ± 0.1	47.8 ± 1.6	18.9 ± 1.1	11.2 ± 2.1	15.7 ± 3.1	2.5 ± 0.2	39.5 ± 0.8	15.7 ± 1.5	34.6 ± 3.7
$\Delta sucT \operatorname{comp}$	1.2 ± 0.3	45.4 ± 2.1	20.4 ± 0.9	12.5 ± 3.9	19.7 ± 1.0	2.2 ± 0.1	39.8 ± 11.5	17.8 ± 4.9	37.9 ± 3.8

<u>Table S6</u>: Glycosyl linkage analysis of per-*O*-methylated mAGP from WT *Mabs* ATCC 19977, the Δ*sucT* mutant and the complemented mutant strain.

Reported values are averages \pm SD of three technical repeats and represent relative distribution in %. No statistically significant differences between strains were observed pursuant to the Student's *t*-test (P < 0.05).

	t-Araf	2-Araf	5-Araf	3,5-Ara <i>f</i>	t-Gal <i>f</i>	5-Galf	6-Galf	5,6-Gal <i>f</i>	Araf/Galf
WT	7.0 ± 0.3	6.0 ± 0.1	36.7 ± 0.9	9.7 ± 0.6	3.7 ± 0.3	20.2 ± 0.9	11.6 ± 0.8	4.5 ± 0.7	1.5 ± 0.1
$\Delta sucT$	7.6 ± 0.8	5.3 ± 0.9	41.5 ± 3.5	8.2 ± 1.4	2.8 ± 0.6	19.1 ± 1.2	11.2 ± 1.1	3.8 ± 0.8	1.7 ± 0.2
$\Delta sucT \operatorname{comp}$	7.2 ± 0.3	5.8 ± 0.2	38.2 ± 0.7	9.5 ± 0.4	3.1 ± 0.2	19.3 ± 0.5	11.4 ± 0.5	4.6 ± 0.4	1.6 ± 0.1

<u>Table S7</u>: Susceptibility of the *Mabs sucT* knock-out mutant to antibiotics and antimicrobial peptides. MIC were determined in cation-adjusted Mueller-Hinton II broth + 0.05% tyloxapol at 37°C and MIC values are given in μ g mL⁻¹. AMK, amikacin; APR, apramycin; AZI, azithromycin; CLA, clarithromycin; ERY, erythromycin; KAN, kanamycin; EMB, ethambutol; RIF, rifampicin; STR, streptomycin; CFX, cefoxitin; TOB, tobramycin; LIN, linezolid; TET, tetracycline; IMI, imipenem; CIP, ciprofloxacin. LL-37 (InvivoGen) and HNP-1 (human α -defensin 1; Bachem) are cationic antimicrobial peptides. MIC determinations were performed two times on independent culture batches. nd, not determined.

Antibiotic	Mabs WT	$Mabs\Delta sucT$	<i>Mabs∆sucT/</i> pMV306- <i>sucT</i>
AMK	10	10	10
APR	2.5	2.5	2.5
AZI	40	80	40
CLA	0.63	0.63	0.63
ERY	5	5	5
KAN	10	10	nd
EMB	>320	>320	>320
RIF	>160	>160	>160
STR	160	160	80
CFX	80	40	80
TOB	40	80	40
LIN	10	5	10
TET	>320	>320	>320
IMI	20	20	20
CIP	5	5	5
LL-37	>100	>100	>100
HNP-1	>100	>100	>100

<u>Table S8</u>: Translocation of *Mabs* WT, $\Delta sucT$ and complemented $\Delta sucT$ mutant strains across polarized monolayers of human A549 lung alveolar type II epithelial cells.

No significant difference in translocation after 24 h was observed between mutant and WT or complemented mutant strains (p>0.05). Statistical analysis using 2-way ANOVA.

	CFU in the upper	CFU recovered in	% of inoculum	Transmembrane
	chamber	the basal chamber	that translocated	potential
		after 24 h	after 24 h	(Ω/cm^2)
WT	$5.6 \pm 0.6 \text{ x} 10^6$	$1.1 \pm 0.8 \text{ x} 10^3$	0.020	254
$\Delta sucT$	$5.0 \pm 0.4 \ x10^{6}$	$8.7 \pm 0.6 \text{ x} 10^2$	0.017	246
$\Delta sucT \operatorname{comp}$	$4.3\pm 0.9\; x10^{6}$	$8.9 \pm 0.5 \ \mathrm{x10^2}$	0.020	251

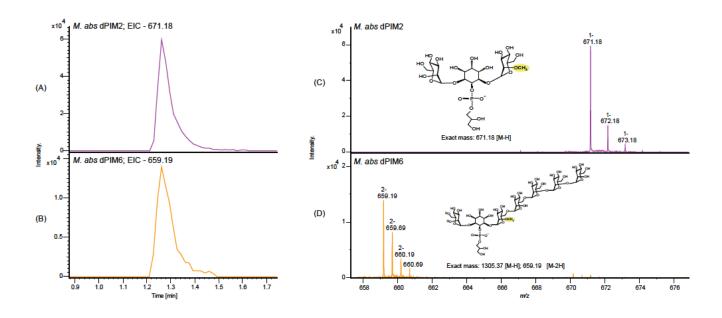


Figure S1: Negative ion liquid chromatography-mass spectrometry (LC-MS) analysis of *Mabs* deacylated PIM₂ (d-PIM₂) and deacylated PIM₆ (d-PIM₆).

(A, B) Extracted ion chromatograms (EICs) for d-PIM₂ (A) and d-PIM₆ (B) with m/z values of 671.18 and 659.19, respectively. (C, D) The mass spectra showing the singly charged [M-H] for d-PIM₂ (C), and doubly charged [M-2H] for d-PIM₆ (D). The chemical structures with exact mass corresponding to the methylated forms of d-PIM₂ (C) and d-PIM₆ (D) are shown.

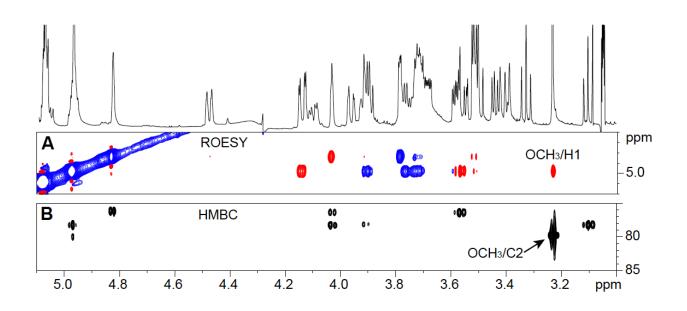
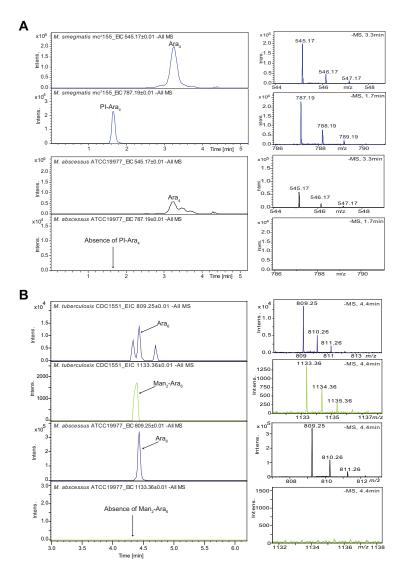


Figure S2: Location of the methyl group on *Mabs* tetra-acylated PIM₂.

Expanded regions of the 2D ¹H-¹H ROESY (δ ¹H 5.10-3.00 and δ ¹H 5.20-4.60) (A) and of the 2D ¹H-¹³C HMBC (δ ¹H: 5.10-3.00, δ ¹³C 85-75) (B) NMR spectra of the Ac₂PIM₂ from WT *Mabs* ATCC 19977 in CDCl₃/CD₃OD/D₂O, 60:35:8 (v/v/v) at 298 K.

On the ROESY spectrum (A), noe contacts are in red, while cosy contacts are in blue. The two important cross peaks allowing the location of the methyl group are annotated.





The most abundant capped digestion product cleaved by *Cellulomonas gelida* endoarabinanase from the nonreducing end of LAM from *M. smegmatis* (A) and *M. tuberculosis* (B) is, respectively, phosphoinositol-Ara₄ with an exact mass of m/z 787.1915 [M-H]⁻ and Man₂-Ara₆ with an exact mass of m/z 1133.36 [M-H]⁻. None of these ions were detected in digested *Mabs* LAM (panels A and B). Three signals at similar retention times with an exact mass of m/z 545.1723 [M-H]⁻ corresponding to Ara₄ were found in the digested *Mabs* LAM which reveals the possibility of more structural isomers of tetraarabinosides released by *Cellulomonas gelida* endoarabinanase.

IN HEL OUT		
Rv1565c MSMEG_3187 MAB_2689	1 MLTLSPPRPPALTPEPALPPVTMGTRTTGFYRHDI DGI RGVATAI VAVFHVWEGRVSGGVDVFI AI 1 PSMGTRKSGFYRHDI DGI RGTATMMVAVFHTWFGRVSGGVDVFI AI 1 MFFVSATKPTKDPEVSPAAAMDATPKPKGDKAFYRYDLDGLRGIAIFLVAVFHVWFGRVSGGVDVFLTL 1	66 46 69 69
Rv1565c MSMEG 3187 MAB_2689	67 SGFFFGGKILRAALNPDLSLSPIAEVIRLIFRLLPALVVVLAGCALLTIAIOPOTRWEAFANOSLASLG 47 SGFFFGGKILRTALDOSTPLRPLSEVVRLVFRLLPALVVVLAAAAVLTILTOPETRWEAFADOSLASLG 70 SGFFYGSKLLRTATTQGASLNPVPVVKRLVFRLLPALILVLAACAVLTVLVQPETRWETFAEQSLASLG 70 ************************************	135 115 138 138
Rv1565c MSMEG 3187 MAB_2689	136 YYONWELASTVSNYI RAGEAVSPI OHTWSMSV0G0FYLAFI LI VAGCAYLI RRLFRGPRAPYI RTMFVV 116 YYONWELANTAADYI RAGETVSPI OHTWSMSV0G0FYTAFI VI TEGEAYLERR 139 YYQNWELANTAADYLAASESVSPLOHLWSMSV0G0FYVGFLALVYLLAVLLRK 139 YYQNWELANTAADYLAASESVSPLOHLWSMSV0G0FYVGFLALVYLLAVLLRK 139 ************************************	204 180 203 207
Rv1565c MSMEG_3187 MAB_2689	205LLSTLTLASFIYAIVAHHAYOATAYYNTFARAWELLAGALVGAVVPHVRWPMWLRTAVATAALAAILSC181LLAALTIASFVYAIIAHNTDOATAYYNSFARAWELLLGALAGALVGFVRWPMWLRTVVSVVSLAAILSC204VIAVLSAASFGYAIYAHLDFQSIAYYNTFARAWELLLGVLVGALVAGTRWPRWLRQLLSFVAVVAILSC208:::::::::::::::::::::::::::::::::	273 249 272 276
Rv1565c MSMEG_3187 MAB_2689	274 GALTDGVKFFPGPWALVPVGATMIMTLAGANROGHPGTRDRIPLPNRILATAPLVALGAMAYSWYLWHW 250 GWFTDGVKFFPGPWALVPVGATTLFTESAANRMSDPRTAGRIPAPNRILATAPEVSLGSMAYSLYLWHW 273 GALINGVREFPGPLALVPVVATLLLILSAANLPADAROPVANRFLATRPLVELGALAYSLYLWHW 277 * :*:***** ***** **:*:*:***** ********	342 318 337 345
Rv1565c MSMEG_3187 MAB_2689	343PLLIFWLSYTGHRHANFVEGAAVLLVSGLLAYLTTRLVEDPLRYRAPAGVRSPAAVPPIPWRLRLRRPT319PLLIFWLSYSGHTAANFVEGAVILLVSGVLAWLTTRYIEEPLRTOPGRAPVPAVPLRARLRRPT338PLLVYWLVYTGEARVTVAEGAAILGISLALAYLTNKYIETPLRYP-RVSPT-SASLWTRLRRPT346***::** *:*.346***::** *:*.	411 382 399 414
Rv1565c MSMEG_3187 MAB_2689	412 TVI GSVVALI I GVALTATSETWREHVTVORAAGKEI SGI SSRDYPGARAL TDHVRVPKI RMRPTVI EVRH 383 TVI GSTVALI GVALTATSETWREHVTVORASGKEI SGI SARDYPGARAL TDHARVPKI PMRPTVI FAKN 400 LALGTSIVLMAVALTATSETWLEHVTVORSNGKELSGLRPRDYPGAGALLYGDRVPDLPMRPTTLEASD 415 :.**: :.*:.***************************	480 451 468 483
Rv1565c MSMEG_3187 MAB_2689	481 DLPTSTKDGCISDFVNPAIINCTYGDVDAPRTIALAGGSHAEHWLTALDLLGRMHHFKVVTYLKMGCPL 452 DIPISTTFGCISDFANVGVINCTYGDKNATRTTALAGGSHAFHWTTALDIIGOKHGFKVVTYLKMGCPI 469 DLPITTEQDCISDFRNRAVITCTYGNPHATRTIALAGGSHAEHWITALDIIGRQHNFKITTYLKMGCPL 484 *** :* :.***** * .:*.******	549 520 537 552
Rv1565c MSMEG_3187 MAB_2689	550 STEEVPLIMGNNAPYPOCHOWVOAAMAKLVADHPDYVFTTSTRPWN-IKPGDVMPATYVGIWOTFADNN 521 TTEEVPLVSGDNRPYPKCHEWNORVMAKLIADHPDYVFTTSTRPWN-IKPGDVMPSTYLGIWETFNENN 538 STEEVPRIAGSNDPYPDCKKWVDEVMSRIIOEHPDYVFTTTTRPRSALGDGDVMPESYLGIWSAFDEAG 553 :***** : *.* ***.*::* : .*:::::::********	617 588 606 621
Rv1565c MSMEG 3187 MAB_2689	618 TPVI AMRDTPWI V-KDGOPFTPADCI AKGGNPOSCGTARSKVI VDRNPTI DEVAREPI I KPI DMSDATC 589 TPVI AMRDTPWI T-RNGKPYEPADCI ADGGDAVSCGVKRSKVI SDRNPTI DYI DREPI MKPI DMSDAVC 607 IPVLGMRDTPWLIDKDGTTYTAADCISAGGTADSCAMDRNRALDDVNPTLAIANREPLLKILDMTRAIC 622 ****.****** ::* :: .** :: ** .**: *:: ** **** ***** ****: ***: *:*	685 656 675 690
Rv1565c MSMEG_3187 MAB_2689	686RTDTCRAVEGNVLVYRDSHHLTPTYMRTMTSELGROIAANTDWW729657REDHCRVVEGNVLLYHDSHHLSATYMRTMTNELGROMAAATGWW700676RPDKCRVVEGNVLVYHDSHHISATYMRTMAKELGROIALATGWWRPAQPGQ726691* * **.******::*****:*****:************	

Figure S4: Primary sequence alignment of SucT from *M. tuberculosis* H37Rv (Rv1565c), *M. smegmatis* mc²155 (MSMEG_3187) and *M. abscessus* ATCC 19977 (MAB_2689) using PSI/TM-Coffee.

Conserved sequence (*), conservative mutations (:), semi-conservative mutations (.), and non-conservative mutations (). Predicted essential functional residues for catalytic activity are boxed in green.

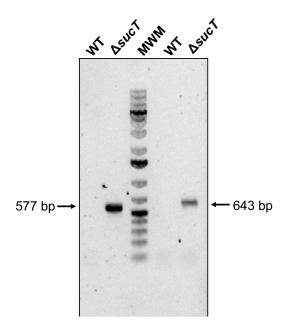
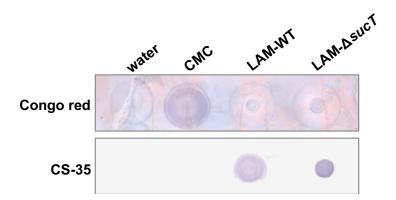


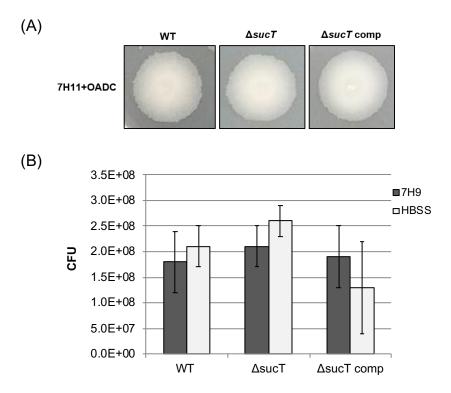
Figure S5: Allelic replacement at the MAB_2689 locus of Mabs ATCC 19977.

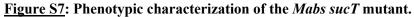
Allelic replacement at the *MAB_2689* locus was confirmed by PCR using sets of primers located outside the allelic exchange substrates and inside the zeocin resistance cassette. The expected sizes of the PCR amplification products in the knock-out mutant are 577-bp for the upstream region of the deleted gene and 643-bp for the downstream region. No amplification was expected in the WT parent strain.



<u>Figure S6</u>: Congo red does not bind to *Mabs* WT and $\Delta sucT$ LAM.

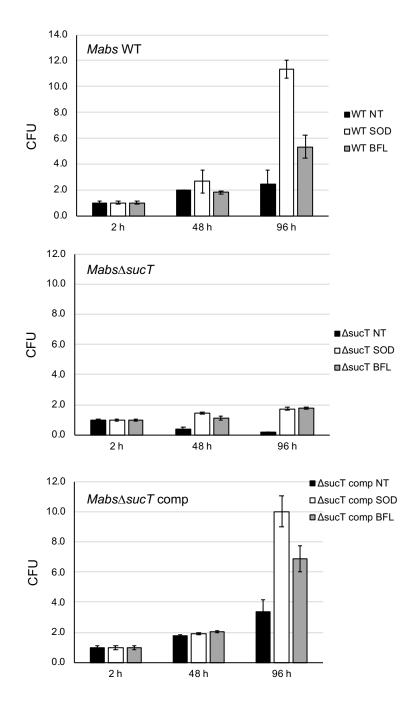
Equal amounts of LAM from the WT and $\Delta sucT$ mutant strains were dot-blotted on the nitrocellulose membrane and stained with Congo red. Carboxymethylcellulose (CMC) (same amount loaded as for LAM) was used as a positive control for Congo red binding. LAM from the WT and mutant strains failed to react with Congo Red while they reacted, as expected, with the anti-LAM antibody CS-35.

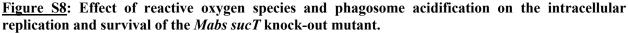




(A) Colony morphology of WT *Mabs* ATCC 19977, the $\Delta sucT$ mutant and the complemented mutant strain after 3 day of incubation on 7H11-OADC agar at 37°C.

(B) Comparison of the ability of WT *Mabs* ATCC 19977, the $\Delta sucT$ mutant and the complemented mutant strain to form static biofilms in Hank's balanced salt solution (HBSS) and 7H9-OADC culture medium. No statistically significant differences in biofilm formation, neither in 7H9-OADC or HBSS buffer, were observed between strains pursuant to Student's *t*-test (P < 0.05).





Infected THP-1 macrophages were either non-treated (NT) or treated with superoxide dismutase (SOD) to inhibit superoxides, or treated with bafilomycin A1 (BFL) to inhibit vacuolar acidification. Intracellular CFU over time (average +/- SD of triplicate wells) were enumerated and are expressed relative to the number of CFU two hours post-infection for each treatment group arbitrarily set to 1. The results presented are representative of three independent experiments.